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24978 7590 03/09/2010

GREER, BURNS & CRAIN
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CHICAGO, IL 60606

EXAMINER	
NASHED, NASHAAT T	
ART UNIT	PAPER NUMBER
1656	

DATE MAILED: 03/09/2010

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/575,505	08/29/2006	Manuel Ferrer	4587.86047	3956

TITLE OF INVENTION: TRANSGENIC ORGANISMS WITH LOWER GROWTH TEMPERATURE

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	\$1510	\$300	\$0	\$1810	06/09/2010

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

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B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail Stop ISSUE FEE**
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24978 7590 03/09/2010

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I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the **Mail Stop ISSUE FEE** address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)

(Signature)

(Date)

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10/575,505	08/29/2006	Manuel Ferrer	4587.86047	3956

TITLE OF INVENTION: TRANSGENIC ORGANISMS WITH LOWER GROWTH TEMPERATURE

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	\$1510	\$300	\$0	\$1810	06/09/2010

EXAMINER	ART UNIT	CLASS-SUBCLASS
NASHED, NASHAATT	1656	435-069100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
 "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): Individual Corporation or other private group entity Government

4a. The following fee(s) are submitted:

Issue Fee
 Publication Fee (No small entity discount permitted)
 Advance Order - # of Copies _____

4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)

A check is enclosed.
 Payment by credit card. Form PTO-2038 is attached.
 The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27. b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

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Date _____

Typed or printed name _____

Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

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ART UNIT		PAPER NUMBER		
1656				DATE MAILED: 03/09/2010

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)

(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 606 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 606 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Notice of Allowability	Application No.	Applicant(s)	
	10/575,505	FERRER ET AL.	
	Examiner	Art Unit	

NASHAAT T. NASHED

1656

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. This communication is responsive to 2/1/10.
2. The allowed claim(s) is/are 32-36, 41, 42 and 44.
3. Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 - a) All b) Some* c) None of the:
 1. Certified copies of the priority documents have been received.
 2. Certified copies of the priority documents have been received in Application No. _____.
 3. Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.

THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

4. A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
5. CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
 - (a) including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
 - 1) hereto or 2) to Paper No./Mail Date _____.
 - (b) including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. Notice of References Cited (PTO-892)
2. Notice of Draftsperson's Patent Drawing Review (PTO-948)
3. Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date _____
4. Examiner's Comment Regarding Requirement for Deposit
of Biological Material
5. Notice of Informal Patent Application
6. Interview Summary (PTO-413),
Paper No./Mail Date _____.
7. Examiner's Amendment/Comment
8. Examiner's Statement of Reasons for Allowance
9. Other _____.

The application is amended as requested in the communication filed February 1, 2010. Accordingly, claims 21-31 are canceled and new claims 31-43 are entered.

Claims 31-43 are pending and under consideration.

The subject matter of claims 37-40 and 43 was not included in claims 21-31 which were subjected restriction requirement, mailed July 27, 2009. Said subject matter is a distinct invention and does not share a special technical feature with the elected subject matter in the response to the restriction requirement, filed August 27, 2009. As indicated in the previous Office action, the special technical feature of the invention of the elected subject matter is the nucleic acid sequence Cpn60 and Cpn10, which were previously known in the prior art. In contrast the special technical feature for the invention of claims 37-40 and 43 is the polypeptide of Cpn60 and Cpn10, which were known in the prior art. Thus, the two inventions are not linked to one another by a special technical feature and therefore, the restriction between the two invention is proper.

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Steve Fallon on February 26, 2010.

The application has been amended as follows:

(I) In the Specification:

Please amend the paragraph on page 5, beginning on line 17, with the following rewritten paragraph:

Figure 7 shows the amino acid sequences expressed from the expression vector coding for the co-expression of native Cpn10 (SEQ ID NO: 10) and the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala of Cpn60 (SEQ ID NO: 11) of *O. antarctica* with the esterase (SEQ ID NO: 12) of *O. antarctica*.

Please amend the paragraph on page 6, beginning on line 1, with the following rewritten paragraph:

Figure 9 shows the amino acid sequences of native Cpn10 (SEQ ID NO: 14) and of the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala of Cpn60 (SEQ ID NO: 15) of *O. antarctica*.

Please amend the paragraph on page 6, beginning on line 4 with the following rewritten paragraph:

Figure 10 shows the DNA sequence of the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala of Cpn60 (SEQ ID NO: 16) of *O. antarctica*.

Please amend the paragraph on page 9, beginning on line 4 with the following rewritten paragraph:

Glu460Ala/Ser462Ala/Val463Ala. 5'-CCT AAC GCA GGT GCT GCA GGG GCA GCG GTT GTT GAT AAA GTG-3' (SEQ ID NO: 27) and 5'-CTC TTT ATC AAC AAC CGC TGC CCC TGC AGC ACC TGC GTT ACC-3' (SEQ ID NO: 28).

Please amend the paragraph on page 9, beginning on line 14 with the following rewritten paragraph:

As a fourth variant, a mutant with three amino acid substitutions was produced as above, introducing the mutations Glu460Ala/Ser462Ala/Val463Ala. This mutant was shown in native PAGE to have a single ring heptameric conformation with an apparent mass of approximately 400 kDa, which corresponds to the wild-type single heptameric ring conformation

Please amend the paragraph on page 9, beginning on line 19 with the following rewritten paragraph:

The above described mutants were purified as described in Example 1. The analysis of the mutant proteins by circular dichroism demonstrated that the triple mutant Glu460Ala/Ser462Ala/Val463Ala as well as the double mutant Lys468Thr/Ser471Gly were not destabilized in their respective overall secondary conformations in comparison to the wild-type Cpn60.

Please amend the paragraph on page 9, beginning on line 24 with the following rewritten paragraph:

Using the measurement of peptide ellipticity at 220 nm to monitor the loss of secondary structure due to increasing temperature, it could be demonstrated that the stabilized double ring mutant Lys468Thr/Ser471Gly has an increased temperature stability at 45 – 55 °C and a rate for ATP hydrolysis 1.3 to 1.6 times higher than the wild type and the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala, the latter having temperature stability up to 24 – 28 °C.

Please amend the paragraph on page 10, beginning on line 24 with the following rewritten paragraph:

When testing the refolding activity of the wild type Cpn60, the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala, and the stabilized double ring mutant Lys468Thr/Ser471Gly under the same conditions with added Cpn10 and ATP (1 mM), it was found that the stabilized single ring mutant catalysed refolding at 4 to 8 °C at 70 – 80%, but was inactive for refolding at above 10 °C. The behaviour of the stabilized single ring mutant led to the conclusion that at the low temperature, the co-chaperonin Cpn10 could bind even to the heptameric conformation, whereas at elevated temperature presumably Cpn10 could not be released from this heptameric single ring.

Please amend the paragraph on page 12, beginning on line 2 with the following rewritten paragraph:

The effect of the presence of a gene product coding for the wild type chaperone from a psychrophilic organism as well as of variant chaperones thereof have been assessed for the growth of *E. coli* at varying temperatures. *E. coli* have been transformed with a plasmid bearing, under the control of an IPTG inducible lac promoter the gene for wild type chaperonin Cpn60 and its co-chaperonin Cpn10 of *O. antarctica*, the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala, and the stabilized double ring mutant Lys468Thr/Ser471Gly, respectively. As shown in Figure 9, *E. coli* without heterologous chaperone grew at 15 °C only to some extent (OD₆₀₀ after 48 h incubation 0.74 +/- 0.24), at 4 °C, no growth was observed. Only *E. coli* expressing the wild type chaperonin Cpn60 and Cpn10 or the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala grew at 4 °C up to an OD₆₀₀ = 1.5 +/- 0.14 after 48 h.. However, at 15 °C, the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala (OD₆₀₀ = 0.75 +/- 0.10) did not enhance viability, but wild type Cpn60 and Cpn10 (OD₆₀₀ = 1.45 +/- 0.12) and the stabilized double ring mutant Lys468Thr/Ser471Gly (OD₆₀₀ = 1.63 +/- 0.24) allowed for an enhanced growth.

Please amend the paragraph on page 13, beginning on line 20 with the following rewritten paragraph:

For expression experiments of the thermo sensitive esterase as cloned above, the esterase gene (est) was cloned into an *E. coli* expression vector under the control of a lac-promoter, alternatively in combination with an expression cassette under a lac-promoter of the wild type chaperonin Cpn60 and its co-chaperonin Cpn10 (cpn10::cpn60::est, see Figures 5 and 6 for amino acid and DNA sequences, respectively) and in combination with the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala and Cpn10 (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala ::est, see Figures 7 and 8 for amino acid and DNA sequences, respectively), under control from a lac-promoter as well. Standard PCR-cloning procedures with primers designed according to the established gene sequences were used.

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Page 15, line 1, please amend the heading in Table 1 as follows:

Table 1:

Growth temperature {[°C]}	Without additional chaperonin	cpn10::cpn60::est	cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est
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Please amend the paragraph on page 16, beginning on line 5 as follows:

When comparing the different chaperonins expressed, it becomes clear that their structure greatly influences their activity at different temperatures. In detail, the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala was only efficient for production of active esterase at below 10 °C.

Please amend the paragraph on page 16, beginning on line 9 as follows:

At temperatures above 20 °C, the esterase activity was significantly lower for all transformants and it is assumed that this is due to the instability of the esterase at these temperatures. However, when analysing the fluorescence intensity of esterase obtained from cultures at 4 °C and 37 °C for both chaperonin transformants, i.e. wild type cpn10::cpn60::est and cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est, the fluorescence intensity of esterase for each transformant measured for the 4 °C culture were five times higher than those for the 37 °C culture. Therefore, misfolding of the thermo-sensitive esterase due to its expression at 37 °C can practically ruled out but higher fluorescence values for the esterase expressed at 4 °C indicate a better folding state, correlating with a higher specific esterase activity.

(II) Amend the Claims as shown in below:

Claim 32 A process for producing a protein by heterologous expression in a host cell wherein said host cell is selected from a group comprising Gram-positive or Gram-negative bacteria, wherein said host cell contains a gene sequence encoding said heterologous protein, wherein said host cell is cultivated at a temperature of below 25°C and wherein said host cell expresses a DNA sequence encoding a chaperonin selected from a group consisting of Cpn60 according to SEQ ID NO: 2, a stabilized single ring mutant chaperonin of SEQ ID NO: 11, and a stabilized double ring mutant of Cpn60 according to SEQ ID NO: 2 with the mutation of Lys468Thr and [I/]Ser471Gly.

Claim 33 The process of claim 32, wherein the host cell is further contains a DNA sequence encoding a chaperonin Cpn10 according to SEQ ID NO: 1.

Claim 34 The process of claim 32, wherein the heterologous protein is selected from the group consisting of mammalian proteins, bacterial proteins, fungal proteins or yeast proteins, and mutants or fusions thereof.

Claim 35 The process of claim 32, wherein the heterologous protein has enzymatic activity or hormonal activity in its native conformation.

Claim 36 The process of claim 32, wherein the cultivation temperature is 4 to 15°C.

Cancel claims 37-40.

Claim 41 The process of claim 33, wherein the heterologous protein has enzymatic activity or hormonal activity in its native conformation.

Claim 42 The process of claim 33, wherein the cultivation temperature is 4 to 15°C.

Cancel claim 43.

Claim 44 The process of claim 34, wherein the bacterial proteins are mesophilic bacterial proteins.

Claims 32-36, 41, 42, and 44 are allowed.

The Following amendments to Figures 7, 8, and 10 are approved:

(a) In Figure 7, the paragraph beginning on line 1, as follows:
Amino acid sequences expressed from vector pBK1CpnSREst: - the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

(b) In Figure 8, the paragraph beginning on line 4 as follows:
SEQ ID No 13: DNA sequence of vector pBK1CpnSREst: the expression cassette for the co-expression of the stabilized single ring mutant chaperonin with the

esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

(c) In Figure 10, the paragraph beginning on line 2 as follows:

SEQ ID No 16: DNA sequence of the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala:

Bases for the above amendment to the specification, the claims, and the drawing regarding the numbers of the amino acid residues are found in the amino acid sequence of SEQ ID NO: 11 and SEQ ID NO: 15.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to NASHAAT T. NASHED whose telephone number is (571)272-0934. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath Rao can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Nashaat T. Nashed/
Primary Examiner, Art Unit 1656